



Original Paper

<http://ajol.info/index.php/ijbcs>

<http://indexmedicus.afro.who.int>

Spatio-seasonal distribution of *Anopheles gambiae sensu lato* and dynamics of the Voltage gate sodium channel knock down resistance mutation (*Vgsc-1014F*) in the city of Lomé, Togo

Koffi Mensah AHADJI-DABLA^{1,2*}, Carine NGOAGOUNI³, B. Dominic DERY⁴,
Yawo Georges APÉTOGBO¹, Guillaume Koffivi KETOH¹ and Isabelle Adolé GLITHO¹

¹Department of Zoology, Faculty of Sciences, University of Lomé, Togo.

²Department of Ecology & Evolutionary Biology and Biodiversity Institute, University of Kansas, USA.

³Institut Pasteur from Bangui, Central African Republic.

⁴Kintampo Health Research Centre, Ghana Health Service, Ministry of Health, Ghana.

*Corresponding author; E-mail: km.ahadjidabla@gmail.com

ABSTRACT

Poor planned urbanization sustains the presence of malaria vectors in some urban areas. Here, we provide information on the spatial and seasonal distribution of *Anopheles gambiae sensu lato* and the dynamics of *kdr* mutation in Lomé, the capital city of Togo. *Anopheles* Larvae were collected in Lomé from January to December 2005, to assess their frequency and abundance. Susceptibility tests were performed from 2006 to 2009 on adults of *Anopheles gambiae s.l.* that emerged from the larvae collected at Akodesséwa following the WHO standard protocol, using DDT, deltamethrin, and permethrin. A molecular assessment of pyrethroid knockdown resistance (*Vgsc-1014F kdr*) was also conducted. In 2005, a total of 2,397 *Anopheles gambiae s.l.* larvae were collected. Out of 124 adults identified by PCR, 92.7% were *An. coluzzii/An. gambiae* and 13.2% were *An. arabiensis*. *Anopheles coluzzii* was more frequent throughout the year regardless the season whereas *An. gambiae* and *An. arabiensis* were more specific to the rainy seasons. A decrease of susceptibility was observed in *An. gambiae s.l.* during the 3 rounds. The *Vgsc-1014F kdr* frequency varied from 7.5% to 8.7%. This situation could affect the success of the integrated distribution campaign to provide individuals with ITNs and LLINs in Togo.

© 2019 International Formulae Group. All rights reserved

Keywords: Breeding sites, *Anopheles gambiae s.l.*, insecticide resistance, dynamics, *Vgsc-1014F kdr* mutation.

INTRODUCTION

The control of malaria, one of the endemic diseases in the world, has become a challenge because of the rapid demographic growth of some urban areas (Keiser et al., 2004; Hay et al., 2005). Poorly planned urbanization characterized by inadequate housing conditions, lack of sanitation and inappropriate water drainage system is

considered as one of the major factors contributing to the sustained presence of *Anopheles gambiae* in some urban settings (Hay et al., 2005). According to the World Health Organization (WHO), the global prevalence of malaria was estimated at 219 million cases and 435,000 deaths in 2017 (WHO, 2018). The burden is heavy in the WHO African Region, where an estimated

93% of all malaria related deaths occurred, and children under 5 years of age are most affected (WHO, 2018). In addition to the mortality rate and the loss of productivity due to the illness, malaria has devastating effects on the cognitive development of children who survived the disease and leaves many disabled for life (Idro et al., 2006; Markley and Edmond, 2009). Millions of clinical episodes of malaria occur annually in urban areas, indicating that the epidemiology of this disease is changing (Keiser et al., 2004). In Togo, it was reported that in 2016, 38% of outpatient consultations and 17% of hospital admissions were related to malaria morbidity (PNLP, 2016). In 2010, the percentage of confirmed cases in the city of Lomé was 38% (PNLP, 2010). The 2004 integrated child health campaign which included a free distribution of insecticide treated bed nets (ITNs) was the first antimalarial campaign undertaken on a national scale (Wolkon et al., 2004), followed by the universal coverage through the distribution of Long Lasting Insecticidal treated Nets (LLINs) in 2011 (Stevens et al., 2013).

Mosquito species responsible for the transmission of malaria parasites in Africa, are mainly *Anopheles gambiae* (formerly *An. gambiae* S form), *An. coluzzii* (formerly *An. gambiae* M form) (della Torre et al., 2005; Coetzee et al., 2013), *An. arabiensis*, and *An. funestus* s.s. which are widespread in tropical and subtropical Africa (Sinka et al., 2012; Coetzee et al., 2013). It is also important to underline that the adult behavior and larval biology of these species are different, hence making their control much easier. *An. coluzzii*, *An. gambiae*, and *An. arabiensis* are members of the *An. gambiae* complex, and, studies of the spatial distribution patterns of *An. gambiae* s.l. have shown that *An. arabiensis* is a major malaria vector, particularly in southern Africa, Madagascar, and along the East-West belt fringes of the Sahel (Coetzee et al., 2000; della Torre et al., 2005). This species is present in sympatry with the *An. gambiae* in East Africa, where *An. coluzzii* is absent (della Torre et al., 2005; Santolamazza

et al., 2015), and with both *An. gambiae* and *An. coluzzii* in West Africa (della Torre et al., 2005). Recently, it was reported in Togo that *An. gambiae*, *An. coluzzii*, *An. arabiensis*, and *An. funestus* are the main malaria vectors (Ahadji-Dabla, 2014) and studies showed that in Lomé, both *An. gambiae* and *An. coluzzii* occurred in sympatry with *An. arabiensis* (Amoudji et al., 2019). Although the distribution of *An. arabiensis* is also influenced by eco-climatic variations, the species can now be found invading urban areas possibly because of adapting to human activities/environments (Dabiré et al., 2012).

Pyrethroids are the only group of insecticides recommended for net treatment few days ago (Zaim et al., 2000). Unfortunately, *Anopheles* mosquitoes in many parts of Africa have become resistant to pyrethroids and such situation could jeopardize the success of vector control measures as studies showed a reduction in the susceptibility to insecticides principally to DDT, deltamethrin and permethrin in the *Anopheles* populations of Lomé (Ahadji-Dabla et al., 2014). In Africa, the major foci of pyrethroid resistance are found in the western and central regions, especially in cotton-growing areas where pyrethroids have been applied intensively against cotton pests (Yadouleton et al., 2011). In these areas the predominant mode of resistance is a reduced sensitivity of the target site due to a single mutation in the sodium channel gene (*kdr*) (Donnelly et al., 2009) also termed a voltage gate sodium channel knock down mutation (*Vgsc-1014F kdr*). This mutation has been implicated as the predominant mode of resistance in *An. gambiae* populations (Ranson et al., 2000). The development of resistance to pyrethroids and other insecticides in *An. gambiae* populations has become a serious threat to the effectiveness of malaria vector control (Yadouleton et al., 2010). In urban areas, this resistance is related to the contamination of breeding sites by pesticides [e.g. Decis, a deltamethrin-based insecticide and malathion (organophosphate)] used in crop protection and vector control, and other pollutants like oils and detergents (Diabaté et

al., 2002; Koudou et al., 2005; Akogbéto et al., 2005). In this study, the spatial and seasonal distribution of *An. gambiae s.l.* and their susceptibility status to DDT and pyrethroids were evaluated. The dynamic of the *Vgsc-1014F kdr* mutation was characterized.

MATERIALS AND METHODS

Study area

The study was carried out in Lomé, the capital city of Togo, located on the Gulf of Guinea on the Atlantic Ocean (06°07'30"N; 01°13'34"E). There are three geomorphologic areas in Lomé: i) a low sandy coast, ii) a lagoon depression and iii) a clayey sandy highland. The coastal band is very slender, with a maximum width of 2 km (Addra et al., 1994) and is limited partly in the North by the lagoon depression. The depression (30 km² and 300 m width) is found across the city from West to East. A highland known as "Plateau de Tokoin", with a 10 m cliff, overhangs the lagoon and decreases slightly in height northwards. The height of the highland also decreases from West to East. Lomé is subdivided into five health districts and has two dry seasons (from December to March and from August to September) and two rainy seasons (from April to July and from October to November). Figure 1 presents the data of the rainfall and the temperature on the larvae abundance during the period of the study.

Mosquito sampling

In 2005, *Anopheles* larvae were collected using dipping method in 14 neighborhoods of Lomé. The dissolved electrolytes of the breeding sites' water were determined using Dissolved Solution conductimeter to measure its conductivity ($\mu\text{S}/\text{cm}$). Both the pH and the temperature were also measured. The geographical coordinates of each prospected site in the different neighborhoods were recorded using a Global Positioning System device (GPS Garmin 48) (Table 1) and were geo referenced on a map using ArcView 3.2 software.

Larvae or pupae collected from each site were stored in plastic boxes and transported for rearing in insectaries (30 °C \pm 2

°C; 12H LD and 72 \pm 2% RH). To study the susceptibility status of *Anopheles* population, larvae were collected in Akodesséwa (06°09'23"N; 01°16'02"E) in 3 rounds because of their abundance and frequency throughout the year.

Susceptibility bioassay

The susceptibility bioassay was conducted in compliance with the WHO standard protocol (WHO, 1998), using two to four days old adult females of *Anopheles gambiae s.l.* from Akodesséwa in 2006, 2008, and 2009. The following insecticides were tested: DDT (4%), deltamethrin (0.05%) and permethrin (1%). For each treatment, five test tubes were used: one untreated paper as a control and four treated papers, and an average of 25 F₀ female mosquitoes were introduced into each tube. Females of *An. gambiae s.l.* used in this study were exposed for one hour to insecticide-treated papers and their knockdown rate was recorded every ten minutes. Insecticide susceptibility status of the wild mosquitoes was compared with that of the reference strain "Kisumu", which is fully susceptible to insecticides. Mosquitoes were then transferred to a recovery tube and supplied with 10% glucose solution after 1 h exposure. Their mortality rates were recorded 24 hours post-exposure. Both dead and surviving mosquitoes were stored separately at -20 °C on silica gel for further molecular characterization.

Identification of sibling species and characterization of *Vgsc-1014F kdr* mutation

The morphological identification of the emerged adults was made using the keys of Gillies and De Meillon (1968), and Gillies and Coetzee (1987). Each mosquito identified was then introduced into an Eppendorf tube containing silica gel and stored at -20 °C for PCR analysis. DNA was extracted according to Scott et al. (1993) from 124 legs of individual mosquitoes and processed by PCR following the protocol described by Fanello et al. (2003). Additional PCR assays for the detection of the presence of *Vgsc-1014F kdr* mutation alleles (Martinez Torres et al., 1998)

were done by pooling the survivors and the dead samples after exposure to each of the insecticides. The presence of *Vgsc-1014F kdr* was characterized using Agd1 and Agd2 as common primers and Agd3 and Agd4 as resistant and susceptible primers, respectively: Agd1 (5'-ATA GAT TCC CCG ACC ATG-3'), Agd2 (5'-AGA CAA GGA TGA TGA ACC-3'), Agd3 (5'-AAT TTG CAT TAC TTA CGA CA-3'), and Agd4 (5'-CTG TAG TGA TAG GAA ATT TA-3'). DNA amplification and purification was performed with a Qiagen purification kit, followed by sequencing. Temperature conditions for DNA amplification were: activation step at 94 °C for 3 s followed by 35 cycles at 94 °C for 30 s, a hybridization phase at 55 °C for 30 s and at 72 °C for 10 s with final elongation for 5 min at 72 °C. To distinguish resistant and susceptible in sibling species, the expected band sizes are: 293 bp fragment for the common band, 195 bp for resistant allele and 137 bp for susceptible allele.

The frequency (F) and abundance (A) of *Anopheles* populations were determined in each district. Frequency (F) was calculated using the formula $F = x/n$; where x is the number of months when larvae were found; n is the number of months of prospection (12) in the year. Abundance (A) was calculated using the formula $A = x/n$; where x is the number of larvae collected; n is the total number of larvae collected during the year.

The Abbott's formula was used to correct the mortality rates when they were above 5% in the control batches (Abbott, 1925) and the resistant/susceptible status was determined using WHO criteria (WHO, 1998). There is susceptibility among the mosquito population when their mortality rate is higher than 98% and there is resistance if their mortality rate is less than 80%. Mortality rates between 80-98% suggested possible resistance. Mortality rates and frequency of *kdr* alleles during the three rounds were compared by chi-square test using R software.

Data analysis

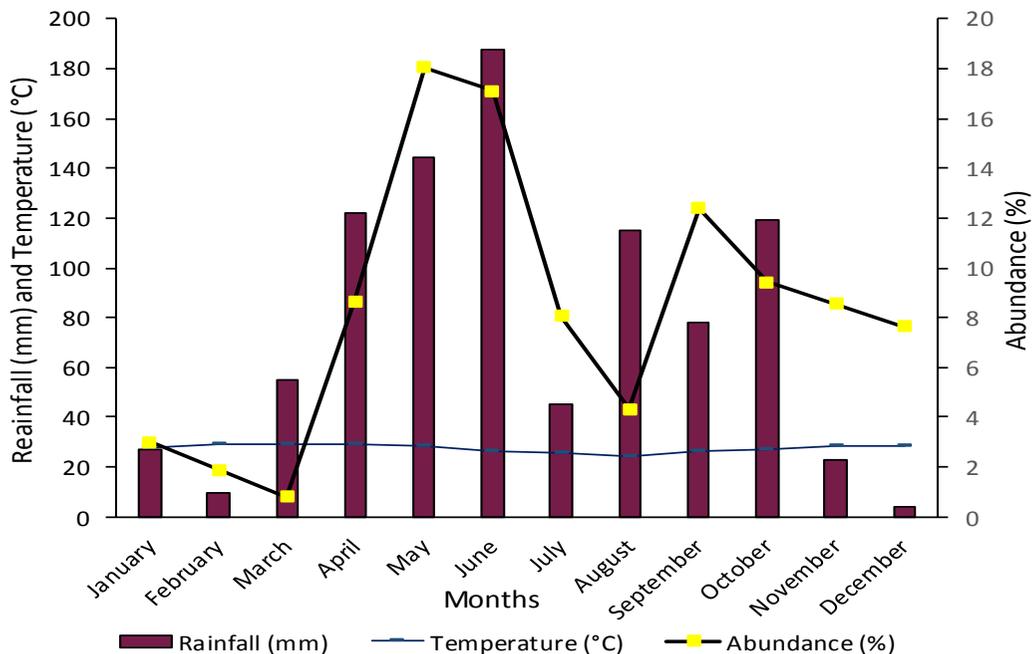


Figure 1: Evolution of temperature and rainfall on larvae abundance.

Table 1: Coordinates, altitude and nature of breeding sites visited in the districts of Lomé.

Districts (Sites)	Nature	Coordinates		Altitude (m)
		Latitude	Longitude	
Adidogomé (In front of Cap Esso)	Pond	06.17809°	01.17583°	44
Agbalépédogan 1	Sandpit	06.19583°	01.20432°	40
Agbalépédogan 2	Sandpit	06.19497°	01.20584°	40
Junction Agbalépédogan – GTA	Puddle	06.19407°	01.20898°	43
North University Campus	Puddle	06.18566°	01.21146°	47
South University Campus 1	Puddle	06.17719°	01.21365°	45
South University Campus 2	Puddle	06.17563°	01.21151°	31
Junction Résidence du Benin	Pond	06.16689°	01.22187°	36
Adéwi-Gbossimé	Pond	06.16285°	01.20937°	33
Junction Atikoumé	Puddle	06.16637°	01.20217°	46
Casablanca-Todman	Pond	06.15968°	01.20174°	35
Junction Collège Protestant	Puddle	06.14568°	01.20472°	36
Junction Tokoin Séminaire	Puddle	06.14798°	01.20889°	41
Dogbéavou	Pond	06.15383°	01.21149°	33
Crossroads Boka (Nyékonakpoè)	Lagoon	06.13716°	01.21062°	24
Boka (Nyékonakpoè)	Lagoon	06.13468°	01.20124°	26
Crossroads Galerie Toutouli	Puddle	06.13043°	01.21216°	26
Crossroads Kodjoviakopé (Traffic lights)	Puddle	06.11792°	01.21176°	30
Traffic circle Central Police station	Puddle	06.13648°	01.22212°	26
Le Togo (Hanoukopé)	Gutter	06.14147°	01.22034°	28
Gendarmerie (Hanoukopé)	Puddle	06.14179°	01.22311°	25
Bè (Community center)	Lagoon	06.14792°	01.24237°	24
Crossroads lagune de Bè (4)	Lagoon	06.14838°	01.24417°	21
Bè (3)	Lagoon	06.15055°	01.25143°	14
Bè (1, 2)	Lagoon	06.15228°	01.25609°	27
	Gravel			
Akodesséwa (stone quarry 1)	washing	06.15624°	01.26759°	29
Akodesséwa gutter	Pond	06.15618°	01.26666°	21
	Gravel			
Akodesséwa (stone quarry 2)	washing	06.15691°	01.26524°	19
Adakpamé	Pond	06.17531°	01.28311°	17
Wuiti-Forever	Pond	06.16106°	01.23169°	34
Crossroads Wuiti (Central Garage)	Puddle	06.15950°	01.22560°	30
Crossroads CICA TOYOTA	Puddle	06.15561°	01.22735°	32
Lycée de Tokoin (enclosure)	Puddle	06.15168°	01.22738°	32
Traffic circle Colombe de la paix	Puddle	06.14949°	01.22988°	29

RESULTS

Location of breeding sites

Ninety-three (93) breeding sites were found positive with the presence of immature mosquito stages in 14 visited (Table 1). Seasons and rainfall influenced the number of positive breeding sites ($Y = 0.06X + 3.6$, $R^2 = 0.6405$). The maximum values of positive breeding sites were obtained in May (16.1%) and June (17.2%) which are the rainiest months of the year.

Abundance and distribution of *Anopheles* populations in Lomé

From January to December 2005; 2,397 larvae were collected. The number of larvae increased with the number of positive breeding sites ($Y = 23.3X + 19.2$, $R^2 = 0.6178$) and with rainfall ($Y = 1.7X + 79.5$, $R^2 = 0.6017$). Thus, more larvae were collected during the rainiest months (Figure 1). The Frequency showed that larvae were frequent in Akodesséwa (92%) than Adidogomé (75%), Bè (54%), and Boka (50%) (Figure 2). In terms of Abundance, more larvae were collected at Akodesséwa (35%) and Adakpamé (25%) both located in the marshy zone (Figure 2). There were two peaks of abundance which occurred during the rainiest month of the long rainy season (June) and of the minor rainy season (October).

From January to December 2005, 124 adults emerged from the *Anopheles* larvae were identified as members of *An. gambiae* complex. Three species were identified in our samples: *An. gambiae* and *An. coluzzii* both represented 92.7%, and *An. arabiensis* represented 7.3%. Figure 3 outlines the spatial distribution of the species. *An. gambiae* and *An. coluzzii* were identified at all the neighborhoods in the southern and northern parts of the lagoon as well as at the marshy and flooded areas of Adakpamé. On the other hand, *An. arabiensis* was collected in some of the neighborhoods located specifically in the northern zone of the lagoon. *An. coluzzii* was found in all collected samples throughout the year and at almost all the neighborhoods whereas *An. arabiensis* was collected only during the rainy seasons. *An. gambiae* was

found particularly during the rainy season in the northern zone of the lagoon, in sympatry with *An. arabiensis* (Figure 3).

An. coluzzii developed under various salinity conditions whereas *An. gambiae* and *An. arabiensis* preferred moderate salinity conditions (Table 2). The highest salinity conditions were found at Bè during the dry season with a maximum concentration of 15000 μS followed by Adakpamé and Akodesséwa. The breeding sites with a concentration of less than 500 μS were more favorable to *An. arabiensis*. In general, larval habitats with $\text{pH} > 7$ were favorable for the development of *Anopheles* larvae.

Resistance status and *Vgsc-1014F kdr* mutation

Figure 4 shows the insecticide resistance status of *An. gambiae s.l.* population collected in Lomé (Akodesséwa) during 3 collection rounds (i.e., 2006, 2008, and 2009). Throughout the surveys, the susceptible strain Kisumu displayed mortality rates not less than 99% for the three insecticides tested. In control groups (untreated papers), the mortality rates of wild *An. gambiae* population were less than 5%. During the three rounds, females of *An. gambiae s.l.* were fully resistant to DDT 4%. In 2006, there was suspected resistance to permethrin 1% and deltamethrin 0.05% with mortality rates of 97.42 and 96.14%, respectively.

A significant decrease in permethrin mortality rates was recorded between 2008 and 2009 ($p < 0.001$). In 2008, *An. gambiae s.l.* was fully susceptible to deltamethrin but in 2009, susceptibility decreased ($p < 0.001$). Though the frequencies of *kdr* increased during the three collection rounds especially in *An. coluzzii*, the differences were not significant ($p > 0.05$) (Table 3). The frequencies were 0.70, 0.85, and 0.86 in 2006, 2008, and 2009, respectively. In 2006, the total of *An. arabiensis* identified in our samples was fully homozygote susceptible but in 2009, two individuals were homozygote resistant.

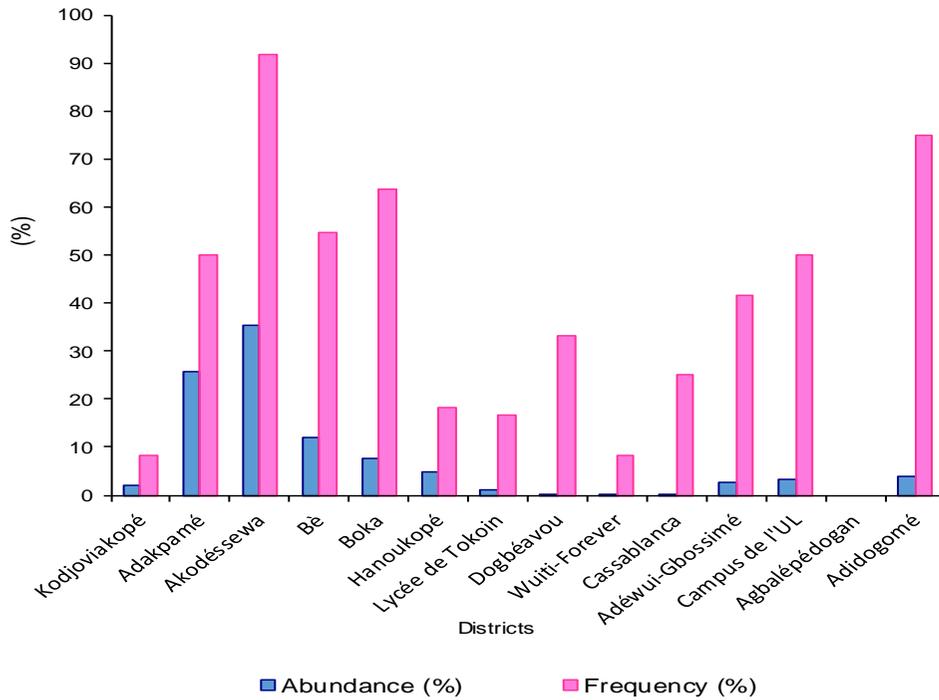


Figure 2: Larvae abundance and frequency by district during the year 2005.

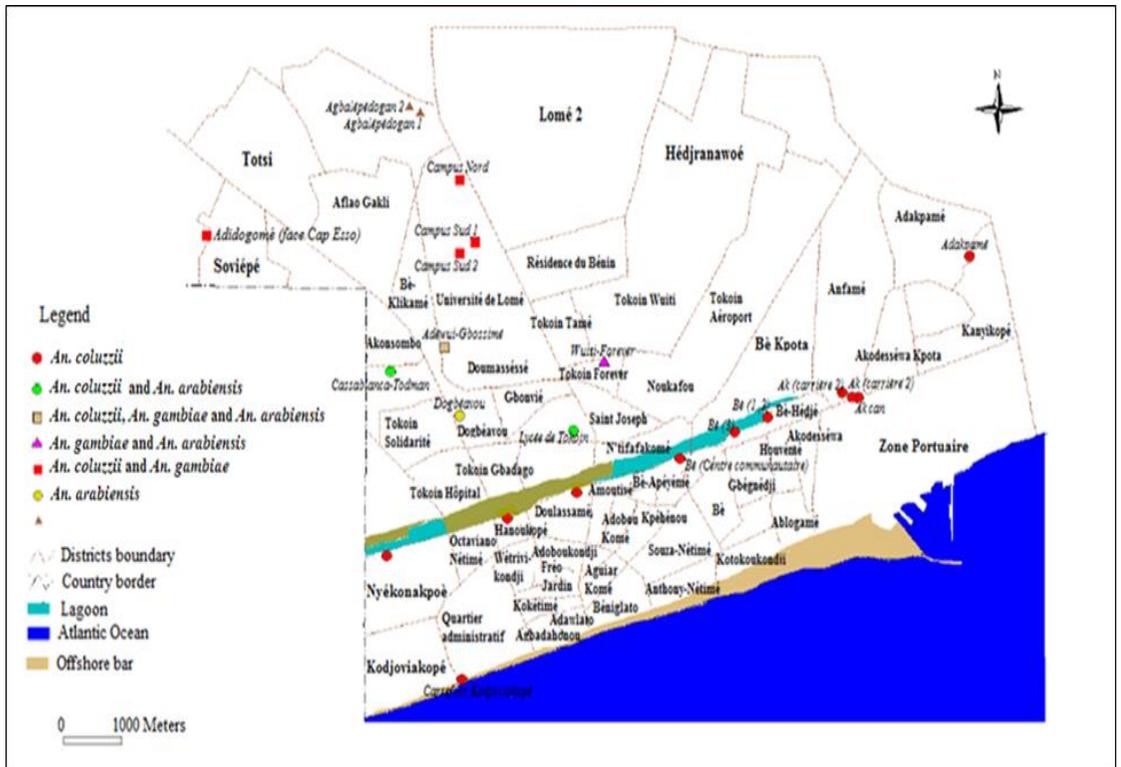


Figure 3: Spatial distribution of *An. coluzzii*, *An. gambiae*, and *An. arabiensis* in Lomé.

Table 2: Physico-chemical properties of the mosquito breeding sites.

Conductivity (µS/cm)	Temperature (°C) (min-max)	pH	Number of larvae collected	Species found	Site
100-500	26-38	<7	1	<i>An. arabiensis</i>	2*, 4*, 5, 9, 10
	28-37	>7	293	<i>An. coluzzii</i> , <i>An. gambiae</i> , <i>An. arabiensis</i>	13
501-1000	26-36	<7	0	-	2, 4*, 5, 7, 9, 13
	28-38	>7	204	<i>An. coluzzii</i> , <i>An. gambiae</i>	
1001-5000	27-42	<7	62	<i>An. coluzzii</i>	1, 2, 4*, 5, 6, 7
	27-42	>7	1424	<i>An. coluzzii</i>	10*, 12, 13
5001-10000	28-39	<7	0	-	1, 5, 6, 10, 11
	30-41	>7	405	<i>An. coluzzii</i>	13
10001-15000	29-37	<7	0	-	6, 11
	35-40	>7	8	<i>An. coluzzii</i>	

*: Negative breeding sites

Visited neighborhoods

- | | | |
|--------------------|-----------------------|---------------------|
| 1: Adakpamé | 6: Bè | 11: Hanoukopé |
| 2: Adéwui-Gbossimé | 7: Boka (Nyékonakpoè) | 12: Kodjoviakopé |
| 3: Adidogomé | 8: University of Lomé | 13: Lycée de Tokoin |
| 4: Agbalépédogan | 9: Cassablanca | 14: Wuiti-Forever |
| 5: Akodesséwa | 10: Dogbéavou | |

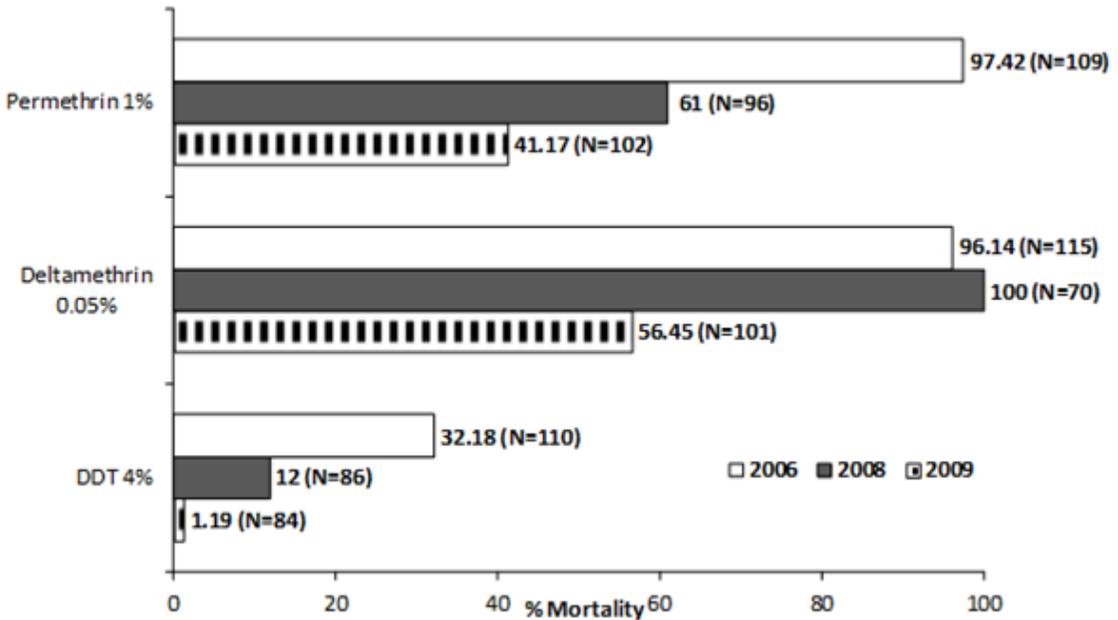


Figure 4: Mortality rates of *An. gambiae s.l.* exposed to DDT and pyrethroids during 3-round collections (2006, 2008, and 2009) in Lomé (Akodesséwa).

Table 3: *Vgsc 1014F kdr* frequencies in *An. gambiae s.l.* per round collection in Lomé.

Periods	Species	n	RR	RS	SS	F (<i>kdr</i>)
June 2006	<i>An. coluzzii</i>	15	10	1	4	0.70
	<i>An. gambiae</i>	5	1	2	2	0.40
	<i>An. arabiensis</i>	10	0	0	10	-
March 2008	<i>An. coluzzii</i>	32	25	5	2	0.85
	Hybrids	1	1	0	0	-
June 2009	<i>An. coluzzii</i>	22	18	2	2	0.86
	<i>An. gambiae</i>	1	0	0	1	0
	<i>An. arabiensis</i>	2	2	0	0	1

DISCUSSION

Anopheles gambiae complex has the most efficient malaria vectors in the world (Coetzee, 2004). Providing baseline information on the distribution and the abundance of the malaria vectors as well as their susceptibility to insecticides is very important for an effective malaria control in Togo. This study showed that in 2005, *Anopheles* larvae were present throughout the year in Lomé, but their frequency and abundance varied depending on the neighborhood and seasons as previously observed in other malaria endemic countries such as Benin (Govoetchan et al., 2014). The presence of larvae was not only related to rainfall because a high number of larvae was collected during the dry season. During dry seasons, larvae were mostly observed and collected in polluted water bodies at Akodesséwa and Bè around the lagoon. The edges and beds of streams serve as *Anopheles* breeding habitats during the months with low precipitation; hence streams can produce large vector populations during the dry seasons as reported by Animut et al. (2012). Larvae were also abundant at Adakpamé in the marshy and flooded zones as well as Adidogomé a poor water draining system area. People in these

neighborhoods are also engaged in low income generating activities due to water availability in these flooded zones. Such activities offer semi-permanent breeding sites for the development of *An. gambiae* mosquitoes. During the rainy seasons, *Anopheles* larvae were collected at all the target neighborhoods in Lomé because of the abundance of breeding sites.

Three sibling species of *An. gambiae s.l.*, namely, *An. gambiae*, *An. coluzzii*, and *An. arabiensis* were found in our samples while Coetzee et al. (2000) have additionally reported the presence of *An. melas* in Lomé. According to Bogh et al. (2003), immediate saltwater concentration has a significant impact on *An. melas*' ability to colonize brackish water larval habitats and to compete with *An. gambiae s.s.* larvae. The absence of *An. melas* could be attributed to the sampling techniques because *An. melas* can also adapt to high salinity conditions which exist in Lomé. *An. gambiae* and *An. coluzzii* were the major species found in all the samples collected in Lomé. They were described as sympatric species (Coetzee et al., 2000). Our results showed that this sympatry was observed mainly during rainy seasons. However, in dry seasons, the species were

present because they mostly breed in anthropogenic breeding sites as reported by della Torre et al. (2005).

Anopheles gambiae and *An. coluzzii* were identified in this study. *An. coluzzii* was predominant over *An. gambiae*, as later found by Ahadji-Dabla et al. (2014) and these findings are similar to the results of studies carried out in Benin (Corbel et al., 2007; Yadouleton et al., 2010; Djègbè et al., 2011) and in some localities in Mali and Nigeria (Wondji et al., 2002; Awolola et al., 2005; Cisse et al., 2015). Indeed, *An. coluzzii* survives better in polluted breeding sites (Wondji et al., 2005) and this could also explain its presence during the dry season whereas *An. gambiae* prefers rainy breeding sites and moderate salinity conditions. The seasonal discrimination of *Anopheles* species in Lomé could also be explained by an intra and interspecific competition. For this purpose, some studies have shown that during dry seasons, the scarcity of *Anopheles* breeding sites leads to a concentration of the species, and under overpopulation conditions, interspecific competition is in favor of *An. coluzzii* (Schneider et al., 2000; Koenraadt et al., 2004). This competition would result in cannibalism which influences the dynamics of the populations of the species of *An. gambiae s.l.* (Koenraadt and Takken, 2003) or would depend on their ability to tolerate the prevailing conditions in their breeding sites particularly the temperature, the level of salinity, the presence of predators and of parasitic or pathogenic agents (Antonio-Nkondjio et al., 2011; Kudom et al., 2012).

The frequency of the *Vgsc-1014F kdr* mutation observed in *An. coluzzii* was comparable to that reported in Benin (Djogbénu et al., 2011; Yadouleton et al., 2011) and in Ghana (Adasi and Hemingway, 2008; Baffour-Awuah et al., 2016). Besides, Djègbè et al. (2011) reported an increase in the frequency of *Vgsc-1014F kdr* mutation especially in *An. coluzzii* in Cotonou, during a four-round survey of dynamics of insecticide resistance in malaria vectors in Benin Republic. In this study, the frequency

increased from 0.70 in 2006 to 0.86 in 2009. This situation could be attributed to the intensive use of insecticide around this area especially for vegetable farming. The high frequency of *kdr* mutation could explain the decrease observed in the susceptibility of *Anopheles* populations in Lomé when exposed to pyrethroids and DDT, as reported by Reimer et al. (2014). However, we can hypothesize that the fully susceptibility observed in 2008 for deltamethrin could be probably due to the sample size (115 in 2006 and 70 in 2008). Our results indicate that populated neighborhoods of Lomé offer favorable conditions for the development of *Anopheles* population the yearlong, especially for *An. gambiae* and *An. coluzzii*. Such conditions are primarily caused by human activities and secondary by rainfall. The sanitary conditions observed in these populated neighborhoods also contribute to the selection of *An. coluzzii*. This study also demonstrated that the resistance dynamics in malaria vectors in one area can change with time, possibly in response to human activities. The increase of the frequency of the *Vgsc-1014F kdr* mutation in these vectors is quite worrying because it could compromise future success of the integrated distribution campaign of LLINs and IRS in Togo. For instance, a complete loss of efficacy of LLIN was recently reported (Riveron et al., 2019). Etang et al. (2016) also recently reported that the intensity of deltamethrin resistance in *An. gambiae s.l.* leads to loss of LLINs bio-efficacy. As such, it is important to set up an evaluation and monitoring scheme for resistance development and a cleaning program in the populated neighborhoods to reduce *Anopheles* populations during the dry season. The results clearly call for further studies to investigate the role of the lagoon in species distribution because *An. gambiae* and *An. arabiensis* were mainly observed in the northern zone of the lagoon. It is important to do a mapping of the resistance dynamics in Lomé by selecting a high number of neighborhoods to allow subsequent follow-ups on the evolution of the *kdr* mutation

within *An. gambiae s.l.* Finally, other types of insecticide resistance mechanisms such as *ace-1^R*, metabolic resistance and/or *Vgsc-1014S kdr* mutation should be investigated.

Conclusion

Our data showed that *Anopheles* larvae were abundant during rainy seasons and were relatively frequent in Akodesséwa and Adidogomé the whole year. *An. coluzzii* was the most present and observed species during the year. The susceptibility in *An. gambiae s.l.* has decreased from 2006 to 2009 with various *kdr* frequencies. The follow-up of the larvae abundance and frequency in one hand and the distribution of *Anopheles* species and their *kdr* dynamics in another hand will help put in place better mosquito control measures, hence contributing to the reduction of malaria transmission in Sub-Saharan Africa.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

KMAD and GKK designed the study, conducted the trial and wrote the manuscript. CN and DBD participated in the field trial and contributed to the writing of the manuscript. YGA helped for the field trial and the laboratory analysis. IAG supervised the study and helped finalizing the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank Drs Fabrice Chandre and Vincent Corbel of Institut de Recherche pour le Développement (IRD), France for their help in molecular identification of the *Anopheles* populations. The first author is grateful to Joseph Chabi for helping in larvae collections and laboratory bioassay and would also like to thank Mr. Kwakuvi WODOKPOE for revising the final version of the manuscript.

REFERENCES

- Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Eco Entomol.*, **18**: 265-267. DOI: <https://doi.org/10.1093/jee/18.2.265a>.
- Adasi K, Hemingway J. 2008. Susceptibility to three pyrethroids and detection of knockdown resistance mutation in Ghanaian *Anopheles gambiae sensu stricto*. *J Vector Ecol.*, **33**: 255-262. DOI: <https://doi.org/10.3376/1081-1710-33.2.255>.
- Addra T, Fahemand AK, Dejong T. 1994. Atlas du développement régional du Togo. PNUD/DTCD/DGPD /DCND, Lomé, Editorg/CARSEUC.
- Ahadji-Dabla KM, Ketoh GK, Nyamador, WS, Apétogbo GY, Glietho IA. 2014. Susceptibility to DDT and pyrethroids, and detection of knockdown resistance mutation in *Anopheles gambiae sensu lato* in Southern Togo. *Int J Biol Chem Sci.*, **8**: 314-323. DOI: <http://dx.doi.org/10.4314/ijbcs.v8i1.27>.
- Ahadji-Dabla KM. 2014. Les vecteurs du paludisme au Togo : Distribution géographique des espèces, Statuts de sensibilité aux insecticides et Perspectives de lutte antivectorielle. Thèse de doctorat, Université de Lomé, Lomé, p. 182.
- Ahadji-Dabla KM, Amoudji AD, Nyamador SW, Apétogbo YG, Chabi J, Glietho IA, Ketoh GK. 2019. High levels of knockdown resistance in *Anopheles coluzzii* and *Anopheles gambiae* (Diptera: Culicidae), major malaria vectors in Togo, West Africa: A 2011 monitoring report. *J Med Entomol.*, tjz030. DOI: 10.1093/jme/tjz030.
- Akogbéto M, Djouaka RF, Kinde-Gazard DA. 2005. Screening of pesticide residues in soils and waters samples from agricultural settings. *Malar J.*, **5**: 22. DOI: <https://doi.org/10.1186/1475-2875-5-22>.
- Amoudji AD, Ahadji-Dabla KM, Hien AS, Apétogbo YG, Yaméogo B, Soma DD, Bamogo R, Atcha- Oubou RT, Dabiré

- RK, Ketoh GK. 2019. Insecticide resistance profiles of *Anopheles gambiae* s.l. in Togo and genetic mechanisms involved, during 3-year survey : is there any need for resistance management ? *Malar. J.*, **18**: 117.
- Animut A, Gebre-Michael T, Balkew M, Lindtjörn B. 2012. Abundance and dynamics of anopheline larvae in a highland malarious area of south-central Ethiopia. *Parasit Vectors*, **5**: 117. DOI: 10.1186/1756-3305-5-117.
- Antonio-Nkondjio C, Fossog BT, Ndo C, Djantio B, Togouet SZ, Awono-Ambene P, Costantini C, Wondji CS, Ranson H. 2011. *Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon): influence of urban agriculture and pollution. *Malar J.*, **10**: 154. DOI: 10.1186/1475-2875-10-154.
- Awolola TS, Oyewole IO, Amajoh CN, Idowu ET, Ajayi MB, Oduola A, Manafa OU, Ibrahim K, Koekemoer LL, Coetzee M. 2005. Distribution of the molecular forms of *Anopheles gambiae* and pyrethroid knock down resistance gene in Nigeria. *Acta Trop.*, **95**: 204-209. DOI: 10.1016/j.actatropica.2005.06.002.
- Baffour-Awuah S, Annan AA, Maiga-Ascofare O, Dieudonné SD, Adjei-Kusi P, Owusu-Dabo E, Obiri-Danso K. Insecticide resistance in malaria vectors in Kumasi, Ghana. *Parasit. Vectors.*, **9**: 633. DOI: 10.1186/s13071-016-1923-5.
- Bogh C, Clarke SE, Jawara M, Thomas CJ, Lindsay SW. 2003. Localized breeding of the *Anopheles gambiae* complex (Diptera: Culicidae) along the River Gambia, West Africa. *Bull Entomol Res.*, **93**: 279-287. DOI: <https://doi.org/10.1079/BER2003239>.
- Cisse MBM, Keita C, Dicko A, Dengela D, Coleman J, Lucas B, Mihigo J, Sadou A, Belemvire A, George K, Fornadel C, Beach R. 2015. Characterizing the insecticide resistance of *Anopheles gambiae* in Mali. *Malar J.*, **14**: 327. DOI 10.1186/s12936-015-0847-4.
- Coetzee M, Craig M, Le Sueur D. 2000. Distribution of the African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitol Today*, **16**: 74-77. DOI: [https://doi.org/10.1016/S0169-4758\(99\)01563-X](https://doi.org/10.1016/S0169-4758(99)01563-X).
- Coetzee M, Hunt RH, Wilkerson R, Della Torre A, Coulibaly MB, Besansky NJ. 2013. *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*, **3619**: 246-274. DOI: <https://doi.org/10.11646/zootaxa.3619.3.2>.
- Coetzee M. 2004. Distribution of the African malaria vectors of the *Anopheles gambiae* complex. *Am J Trop Med Hyg.*, **70**: 103-104. DOI: <https://doi.org/10.4269/ajtmh.2004.70.103>.
- Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbeto M, Hougard JM, Rowland M. 2007. Multiple resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Trop.*, **101**: 207-216. DOI: 10.1016/j.actatropica.2007.01.005.
- Dabiré RK, Namountougou M, Sawadogo SP, Yaro LB, Toe HK, Ouari A, Gouagna L-C, Simard F, Chandre F, Baldet T, Bass C, Diabaté A. 2012. Population dynamics of *Anopheles gambiae* s.l. in Bobo Dioulasso city: bionomics, infection rate and susceptibility to insecticides. *Parasit Vectors*, **5**: 127. DOI: 10.1186/1756-3305-5-127.
- della Torre A, Tu Z, Petrarca V. 2005. On the distribution and genetic differentiation of *Anopheles gambiae* s.s. molecular forms. *Insect Biochem Mol Biol.*, **35**: 755-769. DOI: 10.1016/j.ibmb.2005.02.006.
- Diabaté A, Baldet T, Chandre F, Akogbeto M, Guiguemde TR, Darriet F, Brengues C, Guillet P, Hemingway J, Small GJ, Hougard JM. 2002. The role of Agricultural use of insecticides resistance to pyrethroids in *Anopheles*

- gambiae* s.l. in Burkina Faso. *Am J Trop Med Hyg.*, **67**: 617-622. DOI: <https://doi.org/10.4269/ajtmh.2002.67.617>.
- Djègbè I, Boussari O, Sidick A, Martin T, Ranson H, Chandre F, Akogbéto M, Corbel V. 2011. Dynamics of insecticides resistance in malaria vectors in Benin: first evidence of the presence of L1014S mutation in *Anopheles gambiae* from West Africa. *Malar J.*, **10**: 261. DOI: 10.1186/1475-2875-10-261
- Djogbénou L, Pasteur N, Bio-Bangana S, Baldet T, Irish SR, Akogbéto M, Weill M, Chandre F. 2010. Malaria vectors in the Republic of Benin: Distribution of species and molecular forms of the *Anopheles gambiae* complex. *Acta Trop.*, **114**: 116-122. DOI: 10.1016/j.actatropica.2010.02.001.
- Djogbenou L, Pasteur N, Akogbeto M, Weill M, Chandre F. 2011. Insecticide resistance in the *Anopheles gambiae* complex in Benin: a nationwide survey. *Med Vet Entomol.*, **25**: 256-267. DOI: 10.1111/j.1365-2915.2010.00925.x.
- Donnelly MJ, Corbel V, Weetman D, Wilding CS, Williamson MS, WCt B. 2009. Does kdr genotype predict insecticide-resistance phenotype in mosquitoes? *Trends in Parasitol.*, **25**: 213-219. DOI: 10.1016/j.pt.2009.02.007.
- Etang J, Pennetier C, Piameu M, Bouraima A, Chandre F, Awono-Ambene P, Coosemans M, Corbel V. 2016. When intensity of deltamethrin resistance in *Anopheles gambiae* s.l. leads to loss of Long-Lasting Insecticidal Nets bio-efficacy: a case study in north Cameroon. *Parasit Vectors*, **9**: 132. DOI: 10.1186/s13071-016-1420-x.
- Fanello C, Petrarca V, Della Torre A, Santolamazza F, Dolo G, Coulibaly M, Allouche A, Curtis CF, Touré YT, Coluzzi M. 2003. The pyrethroid knock-down resistance gene in the *Anopheles gambiae* complex in Mali and further indication of incipient speciation within *An. gambiae* s.s. *Insect Mol Biol.*, **12**: 241-245. DOI: <https://doi.org/10.1046/j.1365-2583.2003.00407.x>.
- Gillies MT, Coetzee M. 1987. A supplement to the Anophelinae of Africa South of the Sahara. *Publ S Afr Inst Med Res.*, **55**: 96-110.
- Gillies MT, De Meillon B. 1968. The Anophelinae of Africa South of the Sahara. *Publ S Afr Inst Med Res.*, **54**:1-343.
- Govoetchan R, Gnanguenon V, Ogouwalé E, Oké-Agbo F, Azondékon R, Sovi A, Attolou R, Badirou K, Youssouf RA, Ossè R, Akogbéto M. 2014. Dry season refugia for anopheline larvae and mapping of the seasonal distribution in mosquito larval habitats in Kandi, northeastern Benin. *Parasit Vectors*, **7**: 137. DOI: 10.1186/1756-3305-7-137.
- Hay S, Guerra C, Tatem A, Atkinson P, Snow R. 2005. Urbanization, malaria transmission and disease burden in Africa. *Nat Rev Microbiol.*, **3**: 81-90. DOI: 10.1038/nrmicro1069.
- Idro R, Carter JA, Fegan G, Neville BG, Newton CR. 2006. Risk factors for persisting neurological and cognitive impairments following cerebral malaria. *Arch Dis Childhood.*, **91**: 142-148. DOI: 10.1136/adc.2005.077784.
- Keiser J, Utzinger J, Caldas De Castro M, Smith T, Tanner M, Singer B. 2004. Urbanization in Sub-Saharan Africa and Implication for Malaria Control. *Am J Trop Med Hyg.*, **71**: 118-127. DOI: <https://doi.org/10.4269/ajtmh.2004.71.118>.
- Koenraadt CJM, Githeko AK, Takken W. 2004. The effect of rainfall and evapotranspiration on the temporal dynamics of *Anopheles gambiae* s.s. and *Anopheles arabiensis* in Kenya village. *Acta Trop.*, **90**: 141-153. DOI: <https://doi.org/10.1016/j.actatropica.2003.11.007>.
- Koenraadt CJM, Takken W. 2003. Cannibalism and predation among larvae of *Anopheles gambiae* complex. *Med Vet*

- Entomol.*, **17**: 61-66. DOI: <https://doi.org/10.1046/j.1365-2915.2003.00409.x>.
- Koudou BG, Yao T, Doumbia M, Nsanzabana C, Cissé G, Girardin O, Dao D, N'goran EK, Vounatsou P, Bordmann G, Keiser J, Tanner M, Utzinger J. 2005. Malaria transmission dynamics in Central Côte d'Ivoire: the influence of changing patterns of irrigated rice agriculture. *Med Vet Entomol.*, **18**: 1-11. DOI: <https://doi.org/10.1111/j.0269-283X.2005.00530.x>.
- Kudom AA, Mensah BA, Agyemang TK. 2012. Characterization of mosquito larval habitats and assessment of insecticide-resistance status of *Anopheles gambiae sensu lato* in urban areas in southwestern Ghana. *J Vector Ecol.*, **37**: 77-82. DOI: [10.1111/j.1948-7134.2012.00202.x](https://doi.org/10.1111/j.1948-7134.2012.00202.x).
- Markley JD, Edmond MB. 2009. Post-malaria neurological syndrome: a case report and review of the literature. *J Travel Med.*, **16**:424-30. DOI: [10.1111/j.1708-8305.2009.00349.x](https://doi.org/10.1111/j.1708-8305.2009.00349.x).
- Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Bergé JB, Devonshire AL, Guillet P, Pasteur N, Pauron D. 1998. Molecular characterization to pyrethroid knockdown resistance (kdr) in the major vector *Anopheles gambiae s.s.* *Insect Mol Biol.*, **7**: 179-184. DOI: <https://doi.org/10.1046/j.1365-2583.1998.72062.x>.
- PNLP. 2009. Programme National de Lutte contre le Paludisme, Togo. Rapport d'activités.
- PNLP. 2010. Rapport d'activités. Programme National de Lutte contre le Paludisme, Togo.
- PNLP. 2016. Rapport annuel, 2016. Programme National de Lutte contre le Paludisme, Togo.
- Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. 2000. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Mol Biol.*, **9**: 491-497. DOI: <https://doi.org/10.1046/j.1365-2583.2000.00209.x>.
- Reimer L, Fondjo E, Patchoké S, Diallo B, Lee Y, Arash NG, Ndjemai, HN, Atangana J, Traoré SF, Lanzaro G, Cornel AJ. 2008. Relationship between kdr mutation and resistance to pyrethroid and DDT insecticides in natural populations of *Anopheles gambiae*, **45**(2): 260–266. DOI: <https://doi.org/10.1093/jmedent/45.2.260>
- Riveron JM, Huijben S, Tchapga W, Tchouakui M, Wondji MJ, Tchoupo M, Irving H, Cuamba N, Maquina M, Paaajmans K, Wondji CS. 2019. Escalation of pyrethroid resistance in the malaria vector *Anopheles funestus* induces a loss of efficacy of Piperonyl Butoxide–Based Insecticide-Treated Nets in Mozambique. *J. Infect. Dis.*, **jiz139**. DOI: <https://doi.org/10.1093/infdis/jiz139>.
- Santolamazza F, Caputo B, Nwakanma DC, Fanello C, Petrarca V, Conway DJ, Weetman D, Pinto J, Mancini E, della Torre A. 2015. Remarkable diversity of intron-1 of the para voltage-gated sodium channel gene in an *Anopheles gambiae/Anopheles coluzzii* hybrid zone. *Malar J.*, **14**: 9. DOI: [10.1186/s12936-014-0522-1](https://doi.org/10.1186/s12936-014-0522-1).
- Schneider P, Takken W, McCall PJ. 2000. Interspecific competition between sibling species larvae of *Anopheles arabiensis* and *Anopheles gambiae*. *Med Vet Entomol.*, **14**: 165-170. DOI: [10.1046/j.1365-2915.2000.00204.x](https://doi.org/10.1046/j.1365-2915.2000.00204.x).
- Scott JA, Brogdon WG, Collins FH. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg.*, **49**: 520-529. DOI: <https://doi.org/10.4269/ajtmh.1993.49.520>.
- Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP,

- Temperley WH, Gething PW, Kabaria CW, Burkot TR, Harbach RE, Hay SI. 2012. A global map of dominant malaria vectors. *Parasit Vectors*, **5**: 69. DOI: 10.1186/1756-3305-5-69.
- Stevens, ER, Aldridge A, Degbey Y, Pignandi A, Dorkenoo MA, Hugelen-Padin J. 2013. Evaluation of the 2011 long-lasting insecticide treated net distribution for universal coverage in Togo. *Malar J.*, **12**: 162. DOI: 10.1186/1475-2875-12-162.
- WHO. 1998. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Document WHO/CDS/MAL/98.12, Geneva, Switzerland.
- WHO. 2015. World Malaria Report 2015. World Health Organization, Geneva.
- Wolkon A, Vanden Eng JL, Morgah K, Eliades MJ, Thwing J, Terlouw DJ, Takpa V, Dare A, Sodahlon YK, Doumanou Y, Hightower AW, Lama M, Thawani N, Slutsker L, Hawley WA. 2010. Rapid Scale-Up of Long-Lasting Insecticide-Treated Bed Nets through Integration into the National Immunization Program during Child Health Week in Togo, 2004. *Am J Trop Med Hyg.*, **83**: 1014–1019. DOI: 10.4269/ajtmh.2010.09-0599.
- Wondji C, Simard F, Petrarca V, Etang J, Stantolamazza F, Della Torre A, Fontenille D. 2005. Species and populations of *Anopheles gambiae* complex in Cameroun with special emphasis on the chromosomal and molecular forms of *Anopheles gambiae* s.s. *J Med Entomol.*, **42**: 998-1005. DOI: <https://doi.org/10.1093/jmedent/42.6.998>.
- Wondji C, Simard F, Fontenille D. 2002. Evidence for genetic differentiation between the molecular forms M and S within the Forest chromosomal form of *Anopheles gambiae* in an area of sympatry. *Insect Mol Biol.*, **11**: 11–19. DOI: <https://doi.org/10.1046/j.0962-1075.2001.00306.x>.
- Yadouleton A, Martin T, Padonou G, Chandre F, Asidi A, Djogbenou L, Dabiré R, Aïkpon R, Boko M, Glitho I, Akogbeto M. 2011. Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in Northern Benin. *Parasit Vectors*, **4**: 60. DOI: 10.1186/1756-3305-4-60.
- Yadouleton AW, Padonou G, Asidi A, Moiroux, N, Bio-Banganna S, Corbel V, N'guessan R, Gbenou D, Yacoubou I, Gazard K, Akogbeto MC. 2010. Insecticide resistance status in *Anopheles gambiae* in southern Benin. *Malar J*, **9**: 83. DOI: 10.1186/1475-2875-9-83.
- Zaim M, Aitio A, Nakashima N. 2000. Safety of pyrethroid treated mosquito nets. *Med Vet Entomol.*, **14**: 1–5. DOI: <https://doi.org/10.1046/j.1365-2915.2000.00211.x>.